

AMENDMENTS TO THE CLAIMS

1 – 235 (cancelled)

236. (new) A method of producing a heterologous polypeptide, the method comprising:

a) providing a transgenic plant or transgenic cell comprising a recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction,

i) a sequence complementary to a coding sequence for a heterologous polypeptide;

ii) a sequence complementary to an internal ribosome entry site;

iii) a 3' UTR of a first positive strand single-stranded RNA virus;

b) growing the transgenic plant or transgenic cell; and

c) providing a stimulus to the transgenic plant or transgenic cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA.

237. (new) The method of producing a heterologous polypeptide according to claim 236, wherein the transgenic cell is a plant cell.

238. (new) The method of producing a heterologous polypeptide of claim 236, wherein the promoter is selected from the group consisting of a constitutive promoter and an inducible promoter.

239. (new) The method of producing a heterologous polypeptide of claim 236, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

240. (new) The method of producing a heterologous polypeptide of claim 236, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

241. (new) The method of producing a heterologous polypeptide of claim 236, wherein the internal ribosome entry site is selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Cocksackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4G1 mRNA IRES, a Plautia stali intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

242. (new) The method of producing a heterologous polypeptide of claim 236, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

243. (new) The method of producing a heterologous polypeptide of claim 236, wherein the 3' UTR of a first positive strand single-stranded RNA virus is a 3' UTR of a first positive strand single-stranded RNA virus having no DNA stage.

244. (new) The method of producing a heterologous polypeptide of claim 236, further comprising a sequence complementary to an intron.

245. (new) The method of producing a heterologous polypeptide of claim 236, wherein said DNA molecule further comprises a transcription termination signal.

246. (new) The method of producing a heterologous polypeptide of claim 236, wherein the transgenic plant is a dicotyledonous plant.

247. (new) The method of producing a heterologous polypeptide of claim 246, wherein the dicotyledonous plant is a *Nicotiana* plant.

248. (new) The method of producing a heterologous polypeptide of claim 247, wherein the *Nicotiana* plant is a *Nicotiana benthamiana* plant.

249. (new) The method of producing a heterologous polypeptide of claim 236, wherein the providing a stimulus to the transgenic plant or transgenic cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises infecting the transgenic plant or transformed cell with a second positive strand single-stranded RNA virus.

250. (new) The method of producing a heterologous polypeptide of claim 249, wherein the second positive strand single-stranded RNA virus having no DNA stage is selected from the group consisting of a positive strand single-stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

251. (new) The method of producing a heterologous polypeptide in a transgenic plant of claim 249, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

252. (new) The method of producing a heterologous polypeptide of claim 236, wherein the providing a stimulus to the transgenic plant or transgenic cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises transfecting the transgenic plant or transgenic cell with a cDNA of a second positive strand single-stranded RNA virus.

253. (new) The method of producing a heterologous polypeptide in a transgenic plant or transgenic cell of claim 252, wherein the cDNA of a second positive strand single-stranded RNA virus comprises a cDNA encoding an RNA dependent RNA polymerase.

254. (new) The method of producing a heterologous polypeptide of claim 252, wherein the second positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage.

255. (new) The method of producing a heterologous polypeptide of claim 252, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

256. (new) The method of producing a heterologous polypeptide of claim 236, wherein the providing a stimulus to the cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises transfecting the transgenic plant with RNA of a second positive strand single-stranded RNA virus, the RNA comprising at least one sequence encoding a polypeptide component of an RNA virus replication complex.

257. (new) The method of producing a heterologous polypeptide of claim 236, wherein the RNA comprising at least one sequence encoding a polypeptide component of an RNA virus replication complex is an RNA comprising a sequence encoding an RNA-dependent RNA polymerase.

258. (new) The method of producing a heterologous polypeptide of claim 236, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least from about 50:1 to about 10,000:1.

259. (new) The method of producing a heterologous polypeptide of claim 236, wherein said method of producing a heterologous polypeptide in a transgenic plant is used to confer disease resistance to a transgenic plant further comprising conferring resistance to subsequent infection from a second positive strand single-stranded RNA virus.

260. (new) A recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction:

- a) a sequence complementary to a coding sequence for a heterologous polypeptide;
- b) a sequence complementary to an internal ribosome entry site; and
- c) a 3' UTR of a positive strand single-stranded RNA virus.

261. (new) The recombinant DNA molecule of claim 260, wherein the promoter is a selected from the group consisting of a constitutive promoter and an inducible promoter.

262. (new) The recombinant DNA molecule of claim 260, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

263. (new) The recombinant DNA molecule of claim 260, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

264. (new) The recombinant DNA molecule of claim 260, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4G1 mRNA IRES, a *Plautia stali* intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene

IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a *Rhopalosiphum padi* virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

265. (new) The recombinant DNA molecule of claim 260, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

266. (new) The recombinant DNA molecule of claim 260, wherein the 3' UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

267. (new) The recombinant DNA molecule of claim 266, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a bromovirus.

268. (new) The recombinant DNA molecule of claim 260, further comprising a sequence complementary to an intron.

269. (new) The recombinant DNA molecule of claim 260, further comprising a transcription termination signal.

270. (new) A transgenic cell or transgenic plant comprising the recombinant DNA molecule of claim 260.

271. (new) A transgenic plant cell of claim 270.

272. (new) The transgenic plant of claim 270, wherein the transgenic plant is a transgenic dicotyledonous plant.

273. (new) The transgenic dicotyledonous plant of claim 272, wherein the transgenic dicotyledonous plant is a transgenic *Nicotiana* plant.

274. (new) Transgenic seed comprising the recombinant DNA molecule of claim 260.

275. (new) A vector having at least one site for insertion of a recombinant DNA construct for expressing a heterologous polypeptide in a transgenic cell of claim 260 comprising coding sequence of a heterologous polypeptide in an antisense orientation.

276. (new) A vector according to claim 275, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises a recombination site.

277. (new) A vector according to claim 275, wherein the recombination site is selected from the group consisting of a bacteriophage lambda *att* site and a topoisomerase I-based recombination site.

278. (new) A vector according to claim 275, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises at least one restriction enzyme recognition site.

279. (new) A vector according to claim 275, wherein the at least one restriction enzyme recognition site comprises a polylinker.

280. (new) A recombinant RNA molecule comprising, in the 5' to 3' direction:

- a) an RNA sequence comprising a sequence complementary to a coding sequence for a heterologous polypeptide;
- b) a sequence complementary to an internal ribosome entry site; and
- c) a 3' UTR of a positive strand single-stranded RNA virus.

281. (new) The recombinant RNA molecule of claim 280, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

282. (new) The recombinant RNA molecule of claim 280, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES, a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4G1 mRNA IRES, a *Plautia stali* intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx

mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

283. (new) The recombinant RNA molecule of claim 280, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

284. (new) The recombinant RNA molecule of claim 280, wherein the 3' UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

285. (new) The recombinant RNA molecule of claim 284, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a bromovirus.

286. (new) The recombinant RNA molecule of claim 280, further comprising a sequence complementary to an intron.

287. (new) A transgenic cell or transgenic plant comprising the recombinant RNA molecule of claim 280.

288. (new) The transgenic cell of claim 287, wherein the transgenic cell is a transgenic plant cell.

289. (new) The transgenic plant of claim 287, wherein the transgenic plant is a transgenic dicotyledonous plant.

290. (new) The transgenic dicotyledonous plant of claim 289, wherein the transgenic dicotyledonous plant is a transgenic *Nicotiana* plant.

291. (new) The transgenic *Nicotiana* plant of claim 290, wherein the transgenic *Nicotiana* plant is a transgenic *Nicotiana benthamiana* plant.

292. (new) An RNA complement of a recombinant RNA molecule, of claim 280 further comprising the complement comprising, in the 5' to 3' direction:

- a) a sequence complementary to a 3' UTR of a positive strand single-stranded RNA virus;
- b) an internal ribosome entry site; and
- c) an RNA sequence encoding a heterologous polypeptide.

293. (new) The RNA complement of a recombinant RNA molecule of claim 280, wherein the RNA sequence encoding a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

294. (new) A recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell, the recombinant DNA molecule comprising a promoter operably linked, in the 5' to 3' direction, to DNA sequence comprising:

- a) at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation;
- b) a sequence complementary to an internal ribosome entry site; and
- c) a 3' UTR of a positive strand single-stranded RNA virus.

295. (new) The recombinant DNA molecule of claim 294, wherein the promoter is a selected from the group consisting of a constitutive promoter and an inducible promoter.

296. (new) The recombinant DNA molecule of claim 294, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an Nelf-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

297. (new) The recombinant DNA molecule of claim 294, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

298. (new) The recombinant DNA molecule of claim 294, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C

virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4G1 mRNA IRES, a Plautia stali intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

299. (new) The recombinant DNA molecule of claim 294, wherein the 3' UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

300. (new) The recombinant DNA molecule of claim 294, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a bromovirus.

301. (new) The recombinant DNA molecule of claim 294, further comprising a sequence complementary to an intron.

302. (new) The recombinant DNA molecule of claim 294, further comprising a transcription termination signal.

303. (new) The recombinant DNA molecule of claim 294, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises a recombination site.

304. (new) The recombinant DNA molecule of claim 294, wherein the recombination site is selected from the group consisting of a bacteriophage lambda *att* site and a topoisomerase I-based recombination site.

305. (new) The recombinant DNA molecule of claim 294, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises at least one restriction enzyme recognition site.

306. (new) A kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell, the kit comprising a DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell, the DNA molecule comprising a promoter operably linked, in the 5' to 3' direction, to a DNA sequence comprising:

- a) at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation;

- b) a sequence complementary to an internal ribosome entry site; and
- c) a 3' UTR of a positive strand single-stranded RNA virus.